EFFECT OF CITY WASTEWATER ON SOME OIL CROP PLANTS

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

Master of Philosophy

IN

BOTANY

BY

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2004

Dedicated

to

My Parents

Who have been always great source of inspiration for me

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Certificate

This is to certify that Ms. ***Dilshada Tabassum*** *has worked under my supervision for the* ***M. Phil.*** *degree in* ***Botany****. She has fulfilled all conditions required to supplicate the M. Phil. degree. I, therefore, approve that she may submit her dissertation entitled* ***Effect of City Wastewater on Some Oil Crop Plants****. This is an original work and carried out at the* ***Department of Botany, Aligarh Muslim University, Aligarh****. It has not been submitted for any other degree.*

(Dr. ARIF INAM)

ACKNOWLEDGEMENT

In the beginning I thank Almighty **Allah** for the shower of his benedictions.

I am greatly indebted to my supervisor **Prof. Arif Inam** for his sincere guidance and sympathetic supervision, without whom I would not have been able to complete my work.

I am highly grateful to the Chairman, Department of Botany, **Prof. M. Ishrat Hussain Khan** for providing me all the laboratory and academic facilities throughout my work.

I am deeply and sincerely thankful to **Prof. Samiullah**, **Prof. Aqil Ahmad**, **Dr. N.A. Khan**, **Dr. Feroz Mohammad** and **Dr. Mansoor A. Khan** for their valuable suggestions.

I express vote of thanks to my senior lab collegues Dr. Shamsul Hayat, Dr. Afroza Akhtar, Dr. Sheikh Javid, Dr. Qazi Faridu-din, Irfan Ahmad, Shahla Sayeed, Sarvajeet Singh, Shouket H. Shah, Parvaiz M. Lone, Manzar H. Siddiqui and Mohd. Naeem for their active co-operation.

I bestowee my profound sense of gratitude to my friends Saba Azad, Naheed Akhtar, Shaheena Afroz, Bushra Shahroz, Shabeena Sayeed, Barkat Ali, Riffat Basharat, Amodhini K. Shridharan, Rubina Khan and Ulfat Shafi for their moral support.

I am whole heartedly thankful to my parents, my brothers namely Sheikh Mohsin Parvaiz, Sheikh Tanveerul Afaq and Shiekh Raashid Javid for their love, support throughout my whole academic persuit.

The acknowledgement will remain incomplete without mentioning the names of my cousins Altaf Ahmad Sofi and Arshad Basharat for their love and affection.

I am sincerely thankful to Mr. Rais Ahmad Khan for keeping the patience in typing this dissertation without any delay.

Tabassum.
Dilshada Tabassum

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INTRODUCTION

INTRODUCTION

One of the most important contribution of nineteenth century, in experimental physiology, was the discovery that soil fertility and yields can be increased by adding inorganic nutrients to the soil and today the application of fertilizers is the basic feature of agricultural practices. In addition to supplying the nutrients, inorganic fertilizers also change the pH of arable soils, thus making them suitable for the nutrients availability and plant growth (Noggle and Fritz, 1986).

Among the essential nutrients N, P and K are used in large amounts as they are removed by the crops in large quantities (Patnaik, 1987). Nitrogen is an important constituent of aminoacids, amides, nucleoproteins, certain hormones, chlorophylls and is essential for cell division and expansion and therefore, for growth and development. Phosphorus second to nitrogen is the most limiting nutrient for plant growth, is an essential constituent of energy transfer compounds and co-enzymes. Most essential metabolic processes like photosynthesis, respiration and synthesis of fatty acids are also affected by it. Lastly potassium, which is not the part of any plant constituent but is still essential for higher plants because it acts as a co-factor for various enzymes and also helps in stomatal regulation.

Another important factor for the normal growth and development of any plant is the availability of water. It is the principal constituent of the environment in which all the organisms live. It helps in the maintenance of osmotic potential and water uptake plays a vital role in photosynthesis also. In addition to its various roles, water dissolves nutrients and distributes them to cells. Therefore, proper blend of moisture and nutrients is extremely important. Since fresh water resources are depleting day by day and sufficient quantity of water is available in the form of sewage and industrial wastewater, thus to consider this waste product of industrialization and urbanization and to utilize its manurial ingredients, use of wastewater has drawn the attention of farm scientists during the last few decades. In the present study city wastewater comprising the mixture of sewage and industrial water discharged in the same drain was used as it contained sufficient amount of fertilizing ingredients and if used properly for irrigation of crops can either enhance the effect of fertilizer or save the use of inorganic fertilizers at least partly if not completely, thereby serving the twin objectives of fertilizer economy and environmental protection

India is one of the leading oil seed producing country in the world. The oil seed crop, next to food crops, holds a sizeable share of the country's gross cropped area. With the vast and diverse agroclimatic conditions, India has largest number of commercial varieties of oil seeds. The major nine oil seed crops are groundnut, rapeseed-mustard, soyabean, sunflower, sesame, safflower, castor, linseed and niger. Our country has about 19% of the total world's oil seed area and produces about 10% of the worlds oil seed production (Shukla *et al.*, 2001).

Mustard-rapeseed occupies second position next to groundnut among the various oil yielding crops grown in India. Out of total 26.71 million hectares under oil seeds cultivation during 1998-99, mustard-rapeseed occupied 6.60 million hectares (24.7% of total oilseed area). In terms of production, it produced 6.12 million tonnes out of total 25.21 million tonnes of oil seed (22.9% of total oil seed production). It is the major rabi oil seed crop accounting for 59.3% of area and 67.9% of production of total rabi oil seeds (Khan, 2001). Mustard oil is consumed by a large section of population in Northern and Eastern part of the country since ancient times. Its oil and seeds are used as cooking medium, as a preservative in pickles (achars) and a taste maker. Leaves are used as vegetable and oil cakes as cattle feed.

Agriculture is basically a system of harvesting solar energy through photosynthesis. It is the primary source of energy for humans supplying food, feed and fossil fuels. Yield of crop plants ultimately depends on the size and efficiency of this photosynthetic system, so it is the corner stone of crop production. More than 90% of plant dry matter consists of organic compounds such as cellulose, starch, lipids and proteins. The total dry matter production of plants, the biological yield is therefore related to photosynthesis. In plants the main sites of photosynthesis, mature green leaves (source) and the sites of consumption and storage (sinks) are separated from one another, but photosynthetic source cells produce the sugars, which can move symplastically into sieve tubes and at the sink sites these sugars are being either actively partitioned into cell constituents or converted into some other form of storage. It may be pointed out that photosynthetic rate during grain filling period is normally the most important factor to increase grain size and weight, while before grain filling, most of the photosynthates are consumed in vegetative growth or for flower production. (Gardner *et al.*, 1985). So the optimum production of photosynthates are supposed to be the key factor for the future of any crop species in terms of its productivity.

Keeping in view the beneficial use of fertilizer and wastewater in enhancing crop productivity so as to achieve self sufficiency in oil seed production, it was decided to study the feasibility of city wastewater for irrigation on mustard. To achieve this aim two pot experiments were conducted.

Experiment I

To study the effect of city wastewater and GW on *Brassica juncea* L. cv. *Varuna* under four levels of nitrogen, with potassium and phosphorus as uniform basal dose.

Experiment II

To study the effect of city wastewater and GW on *Brassica juncea* L. cv. *Varuna* under four levels of phosphorus with nitrogen and potassium as uniform basal dose.

It may, however be pointed out that only photosynthesis and related parameters were included in this dissertation while the rest of the data will be the part of the Ph.D. thesis.

REVIEW OF LITERATURE

Effect of wastewater on plants

Since wastewater contains good amount of inorganic matter in the form of nitrites, nitrates, phosphates, sulphates and chlorides, which are essential for the growth and development of plants, so using such wastewater for irrigation purpose will be beneficial as:

1. It will lead to safe disposal and will also prevent environmental pollution and health hazards if otherwise thrown in waterbodies.
2. Nutrients present may be used beneficially.
3. It will overcome the shortage of irrigation water.

Knowing fully the utility of wastewater in 1875 Local Government Board Committee in England stated that the best method of disposing off town sewage is by land irrigation, but where this method was impracticable, other methods might be used. Even earlier during the middle of nineteenth century Liebig had pointed out about the fertilizing value of sewage and recommended land treatment as a suitable method of sewage disposal. Similarly extensive work was carried out by Ruddolf Virchow who established sewage farming in Germany. On the same lines in 1865, Milee and Durand clay has done pioneering work in France. While during the early 20^{th} century similar work was also carried out in the United States of America specially in Colorado, Nebraska and California (Mahida, 1981).

In India also wastewater irrigation has been predominant mode of wastewater disposal as reviewed in the following pages. Bahadur and Sharma (1990) at Bareilly studied the effect of industrial effluent on seed germination and early growth of wheat (*Triticum aestivum*) var. UP 115. They reported a decrease of 46.75% in germination percentage. Significant decrease was also observed in root and shoot length. Jabeen and Saxena (1990) at Gorakhpur studied the effect of Sarya distillery and Gorakhpur fertilizer factory effluent on the behaviour of pea (*Pisum sativum*). They reported that concentrations of 5% and 2.5% respectively from both the factories were favourable. They observed increase in dry matter, pigment and protein content and thus concluded that both waters may act as additional source of nutrients if used for irrigation after proper dilution.

Sharma *et al.* (1990) while applying wastewater from Bhilai steel plant, Madhya Pradesh to linseed (*Linum usitatissimum*) in the field and to sesame (*Sesamum indicum*) and French bean (*Phaseolus vulgaris*) in pots reported that the effluent contained 2534, 5741, 31 and 6441 mg l^{-1} of NO_3–N, NH_4–N, phenol and Fe and it decreased plant Ca and Mg concentrations, but increased P concentration. Fe concentration was decreased by wastewater in French bean and sesame but increased in linseed.

In Coimbatore, Swaminathan (1991) observed that diluted effluent from dying factory increased the physiological components of ground nut (*Arachis hypogea*) seedlings whereas pure effluent decreased the amount of physiological components like chlorophyll, carbonate and protein content. Decrease was also found in seed germination and seedling development.

Misra and Behera (1991) studied the effect of paper industry effluent on growth and macromolecule content of rice (*Oryza sativa*) seedlings. The effects were investigated in relation to both concentrations and time of exposure to effluent. There was a decrease in germination percentage, water imbibing capacity, growth, pigments, carbohydrate and protein contents with increase in effluent concentration and time. In their study, the most sensitive macromolecule was protein. During the same year Salgare and Andhyarijina while studying the effect of polluted water from Patalganga on the mineral content of bank vegetation, reported inhibition in inorganic contents like Na, K, Li, Ca, Mg, Fe and P of four selected species, however there was stimulation in chloride content. They also reported that lower stream site of Patalganga near Rasayani as highly polluted in comparison to midstream area near Mohapada.

The upstream site beyond Kohopli from where river originated was not affected by pollution, so was taken as control. Trivedy and Kirpekar (1991) at Karad observed the impact of dairy waste irrigation on growth and mineral composition of *Glycine max* and *Phaseolus mungo*. results showed that 75 to 100% dairy waste came under moderate to high salinity class. They also observed increase in ash. calcium. nitrogen and phosphorus contents of both the crops. Phosphorus content of *Glycine max* increased in 10. 25 and 50% wastewater but declined in 100%. Abasheeva and Revenskii (1992) in Russia studied the effect of clean water or purified wastewater from cellulose and cardboard mills on chemical composition and productivity of *Avena sativa*. *Brassica napus* and peas. the soil used was alluvial meadow or grey forest soil. They reported increase in dry matter and yield on grey forest soils, whereas there was no effect on rape in both soils. However. they didn't report any adverse effect on chemical composition or feed value. Use of wastewater between sowing and flowering increased the concentration of soluble salts especially Na in the soil. Goswami and Naik (1992) while studying the effect of fertilizer effluent on chlorophyll content of *Cyamopsis tetragonoloba* reported an increase in chlorophyll content at 10% wastewater and there was an adverse effect at higher concentration. Virtually a negative correlation existed between the two. Gupta and Nathawat (1992) in Rajasthan while studying the effect of textile factory effluent on germination and seedling growth of pea (*Pisum sativum*) var. RPG-3, observed that with increase in the concentration of the effluent, there was decrease in root and shoot length and total biomass. Effect on root length was much adverse than on shoot length. Pathak *et al.* (1992) at Bhavanagar reported that *Leucaena leucocephala* grew well on soil irrigated with effluents. further effluents from manufacturing plants of Excel India Ltd., should be used for raising agroforestry. Somashekhar *et al.* (1992) at Bangalore observed the effect of distillery effluent on germination and growth of *Vigna sinensis* and *Trigonella foenum*. The concentration of effluent had a direct bearing on the rate and percentage of germination, however biomass showed decreasing tendency with the increase in effluent concentration.

While studying the effect of treated Mathura refinery wastewater on nitrate reductase activity of green gram var. T-44 and K851, Aziz *et al.* (1993a) at Aligarh reported that wastewater stimulated NRA. It was increased from 15-25 DAS and then decreased. The activity was much stimulated in var. T-44 as compared to variety K-851. In 1993b, They also observed the effect of same effluent on the performance of *Lens culinaris* var. K75 and reported an increase in all the vegetative characteristics. They didn't report any increase in the seed yield in year 1990-1991. but in 1991-1992 seed yield was increased by 6.4% as compared to ground water while working on the same source of wastewater. Inam *et al.* (1993) studied seedling emergence of three varieties of triticale and one of wheat. They didn't report any adverse effect on seedling emergence. however among the cultivars, wheat HD2204 showed maximum percentage, and triticale cv. Delfin performed better followed by TL-419 and Driera. Aziz *et al.* (1994) from the same place reported that effluent increased all growth and yield parameters while no significant change was observed in the physico-chemical properties of soil. Samiullah *et al.* (1994) also at Aligarh reported that the same wastewater proved beneficial for the overall performance of wheat crop. Yield was enhanced by 6, 9 and 10% during 1987-88, 1988-89, 1989-90 respectively over control and thus they recommended the use of treated effluent for the cultivation of this crop. Siddiqui *et al.* (1994) observed the response of *Vigna radiata* under the same refinery effluent and reported an adverse effect on shoot length, root length, leaf number, fresh weight, dry weight plant^{-1}, seed number pod^{-1}, 1000 seed weight and seed yield.

In another trial on wheat. Aziz *et al.* (1995) reported an increase in growth parameters. Wheat cultivar HD 3077 performed best. They were of the opinion that refinery wastewater met the irrigation quality requirements. Aziz *et al.* (1996a) while studying long term effects on six crops and agricultural soil reported that wastewater increased seed yield of wheat, triticale, chick pea. green gram. lentil and pigeon pea except that of summer moong. In 1996b, they also studied the growth and yield of berseem (*Trifolium alexandrium*) for two consecutive years and the data revealed that treated wastewater. fertilizer treatment and their interaction improved all growth characteristics. Aziz *et al.* (1998) studied the effect of five levels of irrigation on different parameters of triticale and reported that treated effluent had more nutrients available as compared to ground water. There was a linear increase in all the parameters studied with increase in the frequency of irrigation. Three irrigations with wastewater proved superior over four irrigations with ground water. Contrary to growth and yield, lower protein and carbohydrate contents were observed by them in wastewater. They further conducted field experiments to evaluate the impact of wastewater on physico-chemical properties of soil. growth, yield and quality of *Zea mays* and *Brassica juncea* during the year 1999. Wastewater increased the growth and yield of both crops.

Hayat *et al.* (2000) also at Aligarh studied the effect of the same water on growth and yield of *Brassica juncea*. During their observations. wastewater had higher quantities of nutrients including the heavy metals. Crop grown under this water showed better response as compared to ground water. Among the various varieties, Varuna proved best, however they didn't report any adverse effect on physico-chemical properties of soil.

Kannabiran and Pragasam (1993) at Pondicherry while studying the effect of distillery effluent on seed germination. seedling growth and pigment content of *Vigna mungo* cv. T9 reported that seeds failed to germinate in the effluent. At 75% effluent,radicles emerged out of seeds but further growth was inhibited from third day. At 50% roots were very short and devoid of laterals, whereas at 25% roots showed few laterals. 10% showed slightly lower values than of control, but 1%, 5% and control showed almost similar results. Higher germination percentage. higher values of Chl a. Chl b and total chlorophyll was recorded in 2.5%. However carotenoid was maximum in 5%.

Karunyal *et al.* (1994) at Madurai worked on the impact of tannery wastewater on seed germination of Paddy, Abutilon (*Arachis holosericea*) and white popinac (*Leucaena leucocephala*) and leaf area, biomass and chlorophyll content of 40 day old seedlings of black gram, cow pea, cotton and tomato. Germination of Abutilon, Paddy and white popinac were inhibited by 25% and 50% effluent and prevented by 75% and 100%. Leaf area and biomass of cotton, black gram, cow pea and tomato seedlings were higher than those of control. Protein and chlorophyll content mg g^{-1} fresh weight was also increased by 25%. In their opinion, 25% proved suitable for plant growth and compensated for fertilizers.

Goyal *et al.* (1995) at Hisar reported that application of distillery water upto 160 m^3ha^{-1} to moong grown in pots increased dry matter production and nitrogen and phosphorus uptake, but dry matter decreased markedly with 640 m^3ha^{-1} application. Application of wastewater 320 m^3ha^{-1} increased electrical conductivity of soil about 3 fold. Agarwal and Chaturvedi (1995) while studying the effect of industrial effluent on aging and chlorophyll content of *Triticum aestivum* var. UP262 reported that increased concentration of effluent had adverse effect on Chl a, Chl b and total chlorophyll, however effect was negligible in plants treated with 25% effluent. While studying the effect of sulphur rich oxalic acid industrial wastewater with different levels of phosphorus on yield and quality of *Brassica juncea*, Sawarkar *et al.* (1995) at Jabalpur reported an increase in seed yield with the increase in phosphorus rate and sulphur application (as wastewater) upto 100 ppm. Phosphorus application increased seed phosphorus and oil content. Sulphur application increased seed sulphur content and available sulphur in the soil. Subramani *et al.* (1995) while studying the effect of distillery effluent on *Vigna radiata* reported that effluent used at higher concentrations had deleterious effects on growth and productivity of crop. Umamaheshwara *et al.* (1995) at Nagarjuna Guntur reported that Vijayawada thermal station effluent had no adverse effect on surrounding vegetation. They also reported that both sulphur and chlorophyll content were higher in test plants.

Vazquez-Montiel *et al.* (1995) in Mexico conducted an experiment in which different concentrations of treated effluent from a trickling filter wastewater treatment plant was applied on *Glycine max* and *Zea mays* under controlled conditions of greenhouse. They reported that both the crops responded well to effluent but there were important differences in terms of grain dry matter and nitrogen uptake. It was attributed to timing and amount of effluent applied, with decreasing effluent application nitrogen uptake from the soil and recovery of nitrogen was greater in maize than in soybean under irrigation and observed an improvement in nitrogen utilization when an effluent deficit vegetative stage was followed by full effluent irrigation applied during reproductive period.

At Agra, Arora and Chauhan (1996) reported significant reduction in germination percentage, length and total biomass of all the tested varieties of *Hordeum vulgare* due to tannery effluent. Chiduanpalan *et al.* (1996) observed that diluted chemical industry effluent 10% promoted germination, growth, chlorophyll and protein content and thus might be used for irrigation after this dilution. Khan and Srivastava (1996) at Faizabad reported that molasses, the by product of sugar factories contains nitrogen, phosphorus and potassium, so should be diverted to land for irrigation. During the same year, Srivastava from Gorakhpur reported that sugar distillery contain higher amounts of phosphates, calcium, sulphates, ammonia and total nitrogen, so plants showed moderate tolerance and better biomass as compared to highly concentrated effluents. Sujatha and Gupta (1996) at Chennai observed that tannery effluent was rich in salt contents, some of which served as nutrients and some were toxic.

Baruah and Das (1997) at Guwahati reported that paper mill effluent polluted soil showed delay in seed germination and reduction in final germination percentage to about 12.5% compared to control. Dutta and Boissya (1997) at the same place studied the effect of effluent on germination of *Oryza sativa* L. var. Masuri seeds and results revealed that effluent at higher concentrations inhibited germination and growth of seedlings. In addition, viability of seeds from effluent affected areas was also decreased over control. Jabeen and Abraham (1997) while studying the effect of Hindustan newsprint factory effluent on germination and seedling characters of *Casia tora*, *Casia ocidentalis*, *Vicia faba* and *Vigna sinensis* reported stimulatory effects on most of the parameters studied. Adverse effect on germination and seedling growth was negligible.

Bera and Saha (1998) at Mohanpur reported that seedling growth of rice cv. Jaya and pigeon pea was stimulated under 1%, 5% and 10% respectively indicating greater tolerance of pigeon pea to effluent. Rajaman *et al.* (1998) reported that both press mud and distillery effluent from sugarcane industry enhanced yield of sugarcane and it also saved the phosphorus and potassium fertilizers.

Dutta and Boissya (1999) in Assam noted the effect of paper mill effluent on chlorophyll, leaf area and grain number of *Oryza sativa* L. var. Masuri. They reported that chlorophyll content and leaf area could not be directly correlated in both affected and non-affected rice plants, but leaf area and grains produced were directly proportional to each other in both affected and not affected plants. Ponmurugan and Jayaseelan (1999) at Sivakasi reported that higher concentrations of effluent inhibited root and shoot morphology, Phytomass/biomass accumulation of *Typha angustata*. Prashanthi *et al.* (1999) at Hyderabad showed that dry matter yield and germination percentage of various crops decreased due to accumulation of toxic substances from industrial effluents. From Faizabad, Srivastava and Pandey (1999) reported that chlorophyll content and biomass of *Eichornea crassipes*, *Pistia stratiotes* and *Hydrilla vertillata*, reduced significantly with the increase in fertilizer effluent in treatment duration from 7, 14 and 21 days of exposure. Subramani *et al.* (1999) reported that ceratophyllum when grown on effluent removed toxic pollutants. This biologically treated effluent promoted growth and yield of green gram to some extent.

Arisha ~~*et al.*~~ (2000) in Egypt while studying the effect of sulphur and sewage sludge on *Spinacea oleracea* and *Pisum sativum* reported that sulphur application had no significant effect on growth, productivity and heavy metal content of both plants, and heavy metals in both the plants didn't exceed statutory limits. Baumgartel and Fricke (2000) in Germany using water from starch potato factories recommended its application for cover and cash crops, winter rape, fallows and winter cereals. In Assam, Dutta and Boissya (2000) reported that paper mill effluent reduced number of flowers, number of fertilized flowers per panicle, average length of ear per plant, number of grains per ear, test weight, volume of 1000 grains and moisture percentage of fresh seeds of *Oryza sativa*.

Kumar (2000) observed the effect of periodic watering with Chakia sugar mill effluent on polygenically characters of *Hordeum vulgare* var. IB65 and reported reduction in every parameter studied. In the same year, Kumar *et al.* at Saharanpur noted that *Amaranthus spirosus*, *Parthenium hysterophorus*, *Cannabis sativa* and *Rumex dentatus* showed tolerance to polluted waters of Hindon river whereas *Cyperus rotundus* and *Euphorbia prostrata* showed higher density along the clear water side. In 2000, Murillo *et al.* in Spain studied the response of olive trees to two types of wastewaters and reported the decrease in leaf water potential, stomatal conductance and photosynthetic rate. In England while studying the influence of municipal compost on temperature, water, nutrient status and yield of maize in temperate soils, Naeini and Cook (2000) reported an overall increase in seed yield.

Niwa *et al.* (2000) in Japan studied the effect of carbonized sewage sludge on plant growth and soil improvements and reported an increase in plant dry weight with increase in sewage sludge, but chemical fertilizers resulted in higher yield. Salgare and Acharekar (2000) at Mumbai while studying the effect of pollution of Kalu river on growth performance of five weeds reported that *Polygonum glabnium* collected from Ambivali area showed highest inhibition in total length of different branches. Sedykh and Tarakanov (2000) studied the effect of oil and gas drilling water on germination of seeds of *Pinus sylvestris*. *Picea obovata*. *Populus balsamifera*. *Salix pentandra*. The aqueous fraction of these wastes didn't adversely effect seed germination and sprouting intensity. Inhibiting effect of waste was associated with toxic elements in compressed jelly like fraction or impairment of air regeme in soil.

In 2001, Kanan noted the effect of effluent on *Phaseolus aureus*. *V radiata* and *Pennisetum typhoides*, and found no germination at 100% effluent. however 1% effluent gave highest values for germination percentage, root length and vigour index. Mahankale and Dauore (2001) in Maharashtra studied the influence of chemical fertilizers and polluted water on maize. They reported that owing to over population. industrialization and urbanization there was depletion in natural resources like grazing lands and pasture lands. so it was the time to explore possible ways of excess forage from unit area of land.

Singh *et al.* (2001) reported that some forest trees except Albizia lebbeck were established well in industrial effluent. There was no change in soil pH but percentage organic matter and E.C. of soil were increased. Sundari and Manakarani (2001) while noting the effect of pulp unit wastewater on agriculture reported that partially treated effluent adversely affected the ground water resources, soil fertility, crop production. land value and resulted in death of livestock also.

Heaton *et al.* (2002) observed root growth of *Salix viminalis* and *Eucalyptus nitens* in response to dairy farm pond effluent irrigation and found that spatial distribution was influenced by effluent rate with greater quantities of both fine and coarse roots in top soil horizon with higher effluent rate of 300

m^3ha^{-1} compared to 150 m^3ha^{-1}.

Effect of N, P and K on mustard

Since fertilizers are the important means by which growth and productivity of crops can be enhanced, so in the present study nitrogen, phosphorus and potassium were also applied to mustard. Therefore, some of the recent references have been included in this review. At Kharagpur, Pariahr in 1991 while studying the effect of nitrogen and irrigation on yield of mustard reported that yield and yield components were greater when irrigation was applied at irrigation depth : cumulative pan evaporation (ID : CPE) of 0.6 along with 60 kg N ha^{-1}. Water use efficiency was higher when irrigation was applied at ID : CPE 0.6. Frequent irrigation under 0.8 ID : CPE didn't significantly increase either grain yield or water use efficiency. The significant increase in grain yield was noticed only upto 60 kg N ha^{-1}.

Rana *et al.* (1991) at Delhi noted the effect of irrigation, plant density and nitrogen application on water, yield and yield attributes of *Brassica juncea*. They reported significantly higher yield at irrigations 0.6 IW : CPE with 1,00,000 plants ha^{-1} and 100 kg N ha^{-1}. Irrigation 0.6 IW : CPE increased total consumptive use of water as compared to no irrigation, but water use efficiency was higher in no irrigation over 0.4 and 0.6 IW : CPE. Higher plant population 2,00,000 plants ha^{-1} increased the water use efficiency but decreased the consumptive use.

In 1992, Singh *et al.* at Karnal noted the effect of drain spacing and phosphorus levels on yield, chemical composition and uptake of nutrients by Indian mustard. They observed that with increasing drainage spacing the number of siliquae m^{-2} and seed yield decreased. Application of phosphorus increased seed yield and yield attributes. Concentration of nitrogen, phosphorus and potassium in seeds and stalks decreased with increasing drain spacing but application of phosphorus increased concentration of these nutrients in seeds and stalks.

Dubey *et al.* (1994) at Pawarkheda studied the effect of different nitrogen and sulphur levels on yield, nutrient uptake, quality and economics of *Brassica juncea*. It was reported that 90 kg N and 300 kg S ha^{-1} significantly increased the seed, oil and protein yields and protein content in seed. Higher doses of N decreased oil percentage in seeds, whereas sulphur upto 40 kg ha^{-1} significantly increased it. The highest net returns were obtained from 90 kg N and 40 kg S ha^{-1}. Mohammad (1994) at Aligarh tested the efficacy of leaf applied phosphorus on yield performance of *Brassica juncea* var. Varuna. Its data revealed that spray treatments were significantly efficacious in augmenting most of the yield attributing parameters as well as seed and oil yield by 20% over water sprayed control. Saraswathy and Dharmalingam (1994) in Tamil Nadu suggested the combination of NPK 50 : 20 : 30 kg ha^{-1} to augment the seed yield and seed quality of *Brassica juncea* cv. 85.

At Pantnagar, Shukla and Kumar (1994) while assessing the effect of nitrogen fertilization on dry matter accumulation, N content, N uptake and seed yield of six varieties of *Brassica juncea* reported that dry matter content in leaves of Varuna was significantly lower than other varieties. The total dry matter at 95 day stage in Kranti and Vardan was at par. Seed yields of Vardan, Krishna and Kranti did not differ significantly. N upto 120 kg ha^{-1} had significant effect on dry matter accumulation in different plant parts as well as seed yield.

Yadav *et al.* (1994) in Haryana observed that increase in amount of water and nitrogen fertilizer application increased the leaf water potential, stomatal conductance, light absorption, leaf area index, seed yield and evapotranspiration and decreased the canopy temperature. Water use efficiency was highest for 0.4 ID : CPE and for 60 kg N ha^{-1} in 1986-87 and 90 kg N ha^{-1} in 1987-88. Combination of 0.6 ID : CPE with 60kg N ha^{-1} gave significantly higher seed yield. Leaf area index showed a significant positive linear relationship with evapotranspiration and light absorption and a negative linear relationship with canopy temperature.

Kumar *et al.* (1997) at Ludhiana while studying the leaf area relationship with solar radiation interception and yield of *Brassica juncea* as influenced by plant population and nitrogen, reported that solar radiation interception and dry matter production increased linearly with leaf area index. Maximum values of LAI, SRI and dry matter yield were found at 150 kg N ha^{-1} and plant density of 4,44,000 plants ha^{-1} However highest seed yield was obtained at 125 kg N ha^{-1} and plant density of 2,66,000 to 3,33,000 plants ha^{-1}. Sharma *et al.* (1997) at Jorhat noted the effect of crop geometry and nitrogen on yield and its attributes of *Brassica* sp. Although crop geometry had no significant effect on yield and yield attributes, but nitrogen significantly increased the seed yield and its attributes. While studying the effect of sulphur and phosphorus on yield and their uptake by *Brassica juncea*, Jaggi and Sharma (1997) at Palampur reported that maximum seed 21.5 q ha^{-1} and straw 69 q ha^{-1} were recorded for the treatment combination P 26.2 + S 90 kg ha^{-1}. sulphur and phosphorus uptake by seed and straw and seed + straw significantly increased upto 30 kg S and 26.2 kg P ha^{-1}. Both nutrient elements interacted synergistically in boosting all the parameters under study.

Thakur and Patel (1998) at Rajgarh evaluated the effect of different levels of nitrogen and phosphorus under rainfed conditions on *Brassica juncea*. 40 kg N ha^{-1} significantly increased the seed, oil and protein yield over 20 kg N ha^{-1}. Further increase in N levels i.e. 60 and 80 kg ha^{-1} didn't bring any significant increase. They also noted that 40 kg P_2O_5 ha^{-1} produced significantly higher seed protein and oil yield compared to 20 kg P_2O_5 but 40 kg P_2O_5 ha^{-1} recorded no significant difference in all the three aspects in comparison to 60 kg P_2O_5 ha^{-1}. While working in Himanchal Pradesh Jaggi and Sharma (2000) conducted a pot experiment to identify the major determinants of *B. juncea* yield and to determine the critical concentrations of nutrients and their rates in the plant at flowering stage as diagnostic tools. They reported that critical nitrogen content for dry matter yield of *Brassica juncea* was 2.9% and 3.1% was for the uptake of N, S and P. However critical sulphur content was 0.31% for all these parameters, critical N : S ratio was 11 : 0 for dry matter yield and 9.5 for N, S and P uptake. The critical S : P ratio for obtaining optimal N, S and P uptake were 2.75, 2.58 and 2.13 respectively and for obtaining dry matter yield the ratio was 2.86. N and S content percent and their critical ratios to achieve flowering close to 75% were 3.1, 0.31 and 9.5 respectively at 6 days after the first and 18 days before the last treatment flowered.

At New Delhi, Garnayak (2001) conducted field experiments to study the effect of irrigation and nitrogen on *Brassica carinata* and *Brassica juncea*. *B. carinata* produced 33% more seed and 53% higher biomass than *B. juncea*. *B. carinata* also removed much N, P, K, S, Ca and Mg than *B. juncea*. Nitrogen application upto 120 kg ha^{-1} progressively increased yields and uptake of all the nutrients but increase was significant only upto 80 kg N ha^{-1}.

Meena *et al.* (2001) in Rajasthan while studying the effect of different concentrations of nitrogen, irrigation and hoeing on yield, nutrient content and nutrient uptake by *Brassica juncea* reported that 60 kg N ha^{-1} + 2 irrigations + interculture (hoeing) was best treatment for enhancing productivity of Indian mustard. Mishra and Kurchania (2001) from Jabalpur reported that NPK contents of *Brassica juncea* cv. Pusa Jaikisan decreased with plant age. Nitrogen application at 120 kg ha^{-1} significantly reduced NPK in weeds, however high nitrogen rates increased crop growth and suppressed weed growth. Herbicide application and hand weeding increased the NPK content in the crop. Plant geometry did not influence nutrient content of crop and weeds. Pawar *et al.* (2001) in Maharashtra while observing the effect of irrigation schedules and fertilizers on growth, yield and quality of *Brassica juncea* reported that 0.6 IW : CPE ratio significantly influenced dry matter production, secondary branches and number of siliquae per plant. Maximum seed yield was obtained in 50 kg N + 40 kg P_2O_5 + 40 kg S ha^{-1}.

Dhaka and Kumar (2003) at Hisar conducted an experiment during winter season 1997 and 1998 on loamy soil of Hisar Agricultural University and found significant increase in seed yield and yield attributes upto 80kg N ha^{-1}. moreover integration of 30kg P_2O_5 ha^{-1} alongwith 80kg N ha^{-1} produced significantly higher yields and yield attributes. Application of vermicompost or farm yard manure (FYM) @ 5t ha^{-1} produced statistically similar yields but vermicompost produced better yield as compared to FYM. Vermicompost @ 10t ha^{-1} proved statistically significant with increase in nitrogen application. integration of phosphorus and with the amount of organic matter.

Conclusion

From the above explained review it becomes clear that wastewater can act as a substitute for fertilizer as well as ground water. However it may be pointed out that type of wastewater based on industries, presence of some toxic elements and salts and the concentration of the wastewater should be kept in mind while going for such studies.

MATERIALS AND METHODS

In order to investigate the comparative effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) while considering various doses of nitrogen and phosphorus observations were carried out at vegetative (50 days after sowing) flowering (70 DAS) and fruiting stages (90 DAS) of growth.

For this purpose 2 pot experiments were conducted during the rabi (winter) season of 2002 and 2003 in the net house of the Department of Botany, Aligarh Muslim University, Aligarh. In both the experiments, *Brassica juncea* var. Varuna was studied. Before starting each experiment earthen pots of 12" diameter were filled with farm soil (7kg pot^{-1}). Seeds were procured from IARI New Delhi, which were tested for viability and surface sterilized. But before sowing of these seeds ground water was applied to each pot in order to provide sufficient moisture for proper germination (Table 1).

1. Sampling of soil

Soil samples for each experiment were collected from the pots before sowing which was mixed thoroughly with farmyard manure. Small quantity of this composite soil was taken, oven dried and analysed for following properties (Table 2).

SOIL ANALYSIS

The soil sample was ground by means of mortar and pestle, and then passed through a 2 mm sieve for determining the following properties.

pH

An important chemical property of soil as a medium for plant growth is its pH value, since essential ions that enter into plant are highly dependent upon hydrogen ion concentration of the soil solution. It was determined with the help of pH meter. To 10 g of soil 50 ml of double distilled water (DDW) was added and shaken thoroughly. After 30 minutes pH of the suspension was observed.

Table 1. Experimental scheme of treatments.

Treatment	GW	50% CWW	100% CWW
		Experiment I	
N_0	–	–	–
N_{40} (0.038g kg^{-1} soil)	+	+	+
N_{80} (0.076g kg^{-1} soil)	+	+	+
N_{120} (0.114g kg^{-1} soil)	+	+	+
A uniform basal dose of P_{30} kg ha^{-1} (0.082g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied before sowing.			
		Experiment II	
P_0	–	–	–
P_{20} (0.055g kg^{-1} soil)	+	+	+
P_{40} (0.110g kg^{-1} soil)	+	+	+
P_{60} (0.165g kg^{-1} soil)	+	+	+
A uniform basal dose of N_{80} kg ha^{-1} (0.076g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied before sowing.			

Table 2. Physico-chemical characteristics of soil before sowing. All determinations in mg l^{-1} in 1:5 (soil : water extracts) or as specified.

Determinations	Experiment I	Experiment II
Texture	Sandy loam	Sandy loam
CEC (meq $100g^{-1}$ soil)	3.42	2.93
pH	7.60	7.50
Organic carbon (%)	0.850	0.798
NO_3–N (g kg^{-1} soil)	0.322	0.287
Phosphorus (g kg^{-1} soil)	0.115	0.118
Potassium	16.00	15.00
Calcium	30.90	29.60
Magnesium	18.81	17.86
Sodium	13.32	12.98
Carbonate	18.64	19.48
Bicarbonate	109.76	98.62
Sulphate	16.24	16.92
Chloride	30.99	28.19

Before reading pH meter was calibrated with a standard buffer of known pH (Jackson, 1973).

Cation exchange capacity

CEC of sample was determined by the method of Ganguly (1951). To 10 g soil, 0.2 N HCl (Appendix. p.ii) was added till the soil became acidic. It was shaken for 30 minutes, then filtered and washed with DDW till it became free from chloride ions, which was checked with $AgNO_3$. The residue from filter paper was transferred to beaker and a suspension of known concentration was prepared. It was then treated with 10 ml of standard KCl solution shaken for 30 minutes and left overnight. Then it was titrated with 0.1N NaOH (Appendix, p.iii), using phenolphthalein (Appendix. p.ii) as an indicator and CEC was calculated as follows:

$$\text{CEC (meq } 100\text{g}^{-1}) - \frac{\text{volume of 0.1N NaOH} \times \text{N of NaOH}}{\text{weight of soil sample}}$$

Total organic carbon

It was estimated according to Walkley and Black (1934) method. 2 g of soil was taken in a 500 ml conical flask to which 10 ml of 1 N potassium dichromate solution (Appendix, p.iii) and 20 ml of concentrated H_2SO_4 were added. After shaking for about 2 minutes, the flask was left as such for 30 minutes for the mixture to react. Then 200 ml of DDW, 10 ml of orthophosphoric acid (85%) and 1 ml of diphenyl amine indicator (Appendix. p.i) was added. A deep violet colour was developed which was titrated against 0.5N ferrous ammonium sulphate solution (Appendix, p.ii) till the colour changed to purple and finally green. Simultaneously a blank was also run without soil sample. Percentage of organic carbon was calculated as follows:

$$\text{Percent of organic carbon} = \frac{\text{Blank titre} - \text{Actual titre}}{\text{weight of soil in grams}} \times 0.003 \times 100 \times \text{N}$$

where N is the normality of ferrous ammonium sulphate solution

Nitrate nitrogen

It was estimated according to Ghosh *et al.* (1983). 20 g of soil was shaken continuously with 50 ml of DDW for 1 hour in 100 ml conical flask fitted with a rubber stopper. A pinch of $CaSO_4$ was added and shaken. The contents were then filtered through a Whatman No 1 filter paper. 20 ml clear filterate was transferred to 50 ml porcelain dish and evaporated to dryness on a water bath. After cooling, 3 ml of phenol disulphonic acid (Appendix, p.ii) was added followed by addition of 15 ml of DDW and stirred with a glass rod until the residue was dissolved. After cooling, the contents were washed down into 100 ml volumetric flask. To this 1 : 1 liquid ammonia (Appendix, p.ii) was added slowly with mixing till the solution was alkaline which was indicated by the development of yellow colour due to presence of nitrate. Another 2 ml of ammonia was added and final volume was made upto 100 ml with DDW. The intensity of yellow colour was read at 410 nm on spectrophotometer. For the preparation of standard curve stock solution containing 100 ppm nitrate nitrogen was prepared by dissolving 0.722 g of potassium nitrate in DDW and the final volume was made upto 1 litre. This was diluted to 10 times to give 10 ppm NO_3^-N solution·Aliquots of 2, 5, 10, 15, 20 and 25 ml were evaporated on water bath to dryness in porcelain dishes. After cooling, 3 ml of phenol disulphonic acid was added and yellow colour was read as described above. Similarly a blank was also run.

Phosphorus

To a 2.5 g of soil in a 100 ml conical flask pinch of Draco G_{60} was added followed by 50 ml of Olsen's reagent (Appendix, p.ii). The blank was also run without soil. The flask was shaken for 30 minutes on a shaker and then the contents were filtered through a Whatman No 1 filter paper. In the filtrate, phosphorus was estimated spectrophotometrically by Dickman and Bray's (1940) method. 5 ml of soil extract was pipetted into 25 ml volumetric flask and 5 ml of Dickman and Bray's reagent (Appendix, p.i) was poured drop by drop with constant shaking, till the effervescence due to CO_2 evolution ceased, the inner wall of flask neck was washed with DDW and the contents diluted to about 22 ml. Then 1 ml of stannous chloride solution (Appendix, p.iii) was added and the volume was made upto the mark. The intensity of blue colour was read at 660 nm on spectrophotometer. For standard curve 0.439 g of potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in half litre of DDW. To this 25 ml of 7 N H_2SO_4 (Appendix, p.iii) was added and the volume was made upto 1 litre. With DDW, giving 100 ppm stock solution of phosphorus. From this 2 ppm phosphorus solution was made after 50 time dilution. For the preparation of standard curve different concentrations of phosphorus (1, 2. 3. 4. 5 and 10 ml) of 2 ppm phosphorus solution were taken in 25 ml volumetric flask. To these 5 ml of extracting reagent (Olsen's reagent) was added. The colour was developed by adding Dickman and Bray's reagent and stannous chloride and read at 660 nm. A blank was also run without the sample. The curve was plotted and the amount of phosphorus was calculated from the curve.

Potassium

5 g of soil was shaken with 25 ml of 1N ammonium acetate (Appendix, p.i) for 5 minutes and it was filtered immediately through a Whatman No 1 filter paper. Stock solution of 1000 ppm K was prepared by diluting 1.908 g KCl in 1 litre of DDW. From the stock solution aliquots were diluted in 50 ml volumetric flask with ammonium acetate solution to give 10 to 40 ppm of K. These were read with the help of flame photometer after setting zero for the blank and at 100 for 40 ppm of K. The curve was obtained by plotting the readings against 10. 15, 20. 25. 30. 35 and 40 ppm concentration of K.

Sodium

The determination was carried out directly from the soil extract (1 : 5; Soil : Water) with the help of flame photometer. Standard curve was prepared by taking. 5.845 g of NaCl dissolved in DDW and the volume was made upto one litre, which gave 1000 meq per litre of Na. From this stock solution. dilutions containing (5. 10, 15, 20, 25, 30, 35, 40, 45 and 50 meq Na/l) were prepared. The curve was drawn by plotting the flame photometer readings on the vertical axis against the concentration of Na on horizontal axis. Na in the unknown sample was read from the curve.

Preparation of soil extract for Calcium, Magnesium, Sulphate, Chloride, Carbonate and Bicarbonate

100 g soil was transferred to a 750 ml flask. To this 500 ml DDW was added and flask was shaken for one hour. The contents were poured through Buchner funnel.

Calcium

It was determined according to Chopra and Kanwar (1982). To 25 ml extract 2 to 3 crystals of carbamate and 5 ml of 16% NaOH solution were added. Then it was titrated against 0.01 ~~M~~ N EDTA (Appendix. p.i) using murexide indicator powder (Appendix. p.ii). till the colour changed from orange red to purple.

Magnesium

To a 25 ml extract 5 ml ammonium chloride ammonium hydroxide (Appendix, p.i) buffer was added followed by titration with 0.01M EDTA using Eriochrome Black T (Appendix. p.ii) as an indicator. The colour changed from green to wine red (Chopra and Kanwar. 1982).

Sulphate

It was estimated in soil extract (1 : 5). To 50 ml extract 2.5 ml of conditioning reagent (Appendix. p.i) was added. It was then stirred on a shaker and during shaking small quantity of BaCl was added. it was then read with the help of Nephelometer.

$$SO_4\ (mg\ l^{-1}) = \frac{mg\ SO_4 \times 1000}{ml\ of\ sample}$$

Chloride

It was also estimated in the soil extract (1 : 5). To 50 ml sample 2.0 ml of 5% K_2CrO_4 indicator (Appendix. p.ii) was added. Then it was titrated against 0.02 N silver nitrate (Appendix. p.iii) and calculated.

$$Cl\ (mg\ l^{-1}) = \frac{(ml \times N)\ of\ AgNO_3 \times 1000 \times 35.5}{ml\ sample}$$

Carbonates and bicarbonates

These were estimated according to method of Richards (1954). For the estimation of carbonates, 50 ml of extract (1 : 5) was taken in a conical flask and 5 drops of phenolphthalein indicator was added. Appearance of pink colour indicated the presence of carbonates. It was titrated against 0.01 N H_2SO_4 (Appendix. p.iii) till the solution became colourless. To this colourless extract few drops of methyl orange indicator (Appendix. p.ii) were added. The yellow colour of extract was titrated against 0.01 N H_2SO_4 till the colour changed to rose red.

$$Carbonate\ (meq\ l^{-1}) = (2y) \times normality\ of\ H_2SO_4 \times \frac{1000}{ml\ of\ aliquot}$$

$$= 2y \times 2$$

$$Bicarbonate\ (meq\ l^{-1}) = (z - 2y) \times normality\ of\ H_2SO_4 \times \frac{1000}{ml\ of\ aliquot}$$

$$= z - 2y \times 2$$

where y = reading of burette for titration of carbonates

z = reading of burette for titration of bicarbonates

WATER ANALYSIS

The following parameters were studied (Table 3).

Total solids

A 250 ml unfiltered sample was taken in an evaporating dish and was allowed to evaporate by keeping it on water bath. Total solids were calculated as

$$\text{Total solids } (g\ l^{-1}) = \frac{A - B \times 1000}{V}$$

where A = final weight of dish in g

B = Initial weight of dish in g

V = volume of sample in ml.

Total dissolved solids

A 250 ml filtered sample was taken in an evaporating dish and allowed to evaporate by keeping it on a water bath. It was calculated as follows.

$$\text{Total dissolved solids } (g\ l^{-1}) = \frac{A - B \times 1000}{V}$$

where A = final weight of dish in g

B = Initial weight of dish in g

V = volume of sample in ml.

Total suspended solids (TSS)

TSS were determined by calculating difference between total solids and total dissolved solids.

$$\text{TSS } (g\ l^{-1}) = \text{TS} - \text{TDS}$$

pH

pH was determined with the help of pH meter which was adjusted before use with standard buffer of known pH.

Biological oxygen demand

BOD is widely used to determine the pollution strength of sewage and industrial wastewater in terms of oxygen that micro-organisms would require

Table 3. Chemical characteristics of ground water (GW). 50% city wastewater (50% CWW) and 100% city wastewater (100% CWW). All determinations in mg l^{-1} or as specified.

Determinations	Sampling time								
	10 Nov.			25 Dec.			12 Feb.		
	GW	50% CWW	100% CWW	GW	50% CWW	100% CWW	GW	50% CWW	100% CWW
T.S.	988	1451	2480	971	1393	2234	970	1237	2030
T.D.S.	540	858	1410	532	830	1391	528	741	1296
T.S.S.	448	593	1070	439	563	843	442	496	734
pH	7.8	7.0	6.9	8.0	7.3	7.1	8.1	7.3	7.0
B.O.D.	17.34	76.18	140.32	16.58	70.13	128.12	16.16	63.25	112.33
C.O.D.	42.32	157.34	361.12	38.18	184.28	352.14	38.22	181.24	354.55
NO_3–N	0.80	3.61	8.01	0.76	3.57	7.10	0.74	3.69	6.24
PO_4^-	0.76	1.31	1.90	0.65	1.35	2.01	0.73	1.12	1.61
K	8.39	13.24	19.91	8.09	13.12	19.24	7.82	13.13	19.58
Ca	28.12	85.34	152.48	26.39	81.18	168.16	26.12	89.44	154.43
Mg	18.32	69.42	132.13	16.83	70.14	120.14	17.54	65.18	115.32
Cl	71.42	101.18	136.17	68.21	98.14	128.11	65.37	96.18	132.18
CO_3^-	18.16	48.14	96.84	19.47	54.34	102.42	21.82	51.12	72.41
HCO_3^-	64.16	192.43	374.12	62.18	186.18	351.22	66.12	189.14	366.44

for complete stabilization. Different volume of effluent samples were placed in BOD bottles (250 ml) to get dilution of the samples to obtain the required depletion ranging between 0.1 and 1.0%. These bottles were then filled with DDW, stoppered and one set of bottles was incubated for 5 days in an incubator maintained at 20°C and in other set dissolved oxygen (DO) was determined immediately. The DO of the samples was determined by just adding 2 ml manganous sulphate solution (Appendix, p.ii) followed by 2 ml of alkali azide iodide reagent (Appendix, p.i) by means of graduated pipette by dipping its end well below the surface of the liquid. The BOD bottles were stoppered and mixed well by inverting them. The bottles were stand till the precipitate settled half way leaving a clear supernatant above the manganese hydroxide flocs. The stopper was removed and 2 ml of H_2SO_4 was immediately added. Each bottle was restoppered and the contents were mixed by gentle inversion, until dissolution was complete. 200 ml of sample was taken in a 500 ml conical flask, then 2 ml starch indicator (Appendix, p.iii) was added and titrated against 0.025 N sodium thiosulphate (Appendix, p.iii) till the disappearance of blue colour. The reading of sodium thiosulphate used up was indicative of DO of the sample in mg/l. BOD was calculated using the following relationship.

$$\text{BOD (mg l}^{-1}\text{)} = \frac{D_1 - D_2}{P}$$

Where D_1 and D_2 are dissolved oxygen of diluted samples 15 minutes after the preparation of sample and after 5 days of incubation respectively and P is the decimal fraction of the sample used.

Chemical oxygen demand

COD is a measure of oxygen equivalent of that proportion of organic matter in a sample, which is susceptible to oxidation by a strong chemical oxidant. 0.4 g of mercuric sulphate was placed in a refluxing flask and 20 ml sample was added. Both were mixed well and 10 ml of 0.25 N standard potassium dichromate solution (Appendix, p.iii) was added followed by 30 ml sulphuric acid and small amount of silver sulphate. A blank was run using DDW instead of the sample. These were subjected to reflux for 2 hours, cooled and then diluted to about 100 ml with DDW. The contents were then titrated against standard ferrous ammonium sulphate 0.1 N solution (Appendix, p.iii). The COD was calculated by following relationship.

$$\text{COD (mg l}^{-1}\text{)} = \frac{A - B \times N \times 8000}{\text{ml of sample}}$$

where A = ml of ferrous ammonium sulphate used for blank titration

B = ml of ferrous ammonium sulphate used for sample titration

N = Normality of ferrous ammonium sulphate

Nitrate nitrogen

First nitrate standard was prepared in the range of 0.1 to 1 mg l^{-1} N by diluting 1, 2, 4, 7 and 10 ml standard nitrate solution to 10 ml with DDW. Residual chlorine in the samples was removed by adding 1 drop sodium arsenite solution for each 0.1 mg Cl and mixed. One drop was added in excess to 50 ml portion. For colour development a number of reaction tubes were set in a wire rack. To each tube 10 ml sample was added. The rack was then placed in a cool water bath and 2 ml NaCl solution was added and mixed well. Then 10 ml H_2SO_4 was mixed and cooled. 0.5 ml brucine sulfanilic acid (Appendix, p.i) reagent was added and tubes swirled to mix and then placed in a water bath not less than 95°C. After 20 minutes it was taken out and cooled in a cold water bath. Reading was taken against reagent blank at 410 nm with spectrophotometer.

Standard curve was prepared from the absorbance value of standard run together with the sample and correlating by subtracting their "sample blank" values from their final absorbance values. The concentration of NO_3^- N was read directly from the standard curve.

Phosphate

To a 100 ml sample containing not more than 0.2 mg phosphorus and free from colour and turbidity, 0.05 ml phenolphthalein indicator was added. If the sample turned pink strong acid solution was added dropwise to discharge the colour. Small sample was taken and diluted to 1000 ml with DDW. After discharging the pink colour with acid, 4 ml ammonium molybdate reagent (Appendix, p.i) was added. After 10 minutes the colour was measured spectrophotometrically at 690 nm and comparison with calibration curve was made using a DDW blank.

$$\text{Phosphate (mg l}^{-1}\text{)} = \frac{\text{mg P} \times 1000}{\text{ml of sample}}$$

Potassium

The estimation of potassium was carried out directly with flame photometer at 768 nm using appropriate filter and a standard curve by taking known concentration of potassium. A stock solution of 1000 ppm K was prepared by dissolving 1.908 g KCl in 1 litre DDW. Dilute solutions containing 2, 5, 10, 15, 25 ppm were prepared from stock solution. The standard curve was prepared by plotting photometer (Systronics) readings against concentrations of K.

Calcium

50 ml water sample was taken in a conical flask and neutralized with acid. It was boiled for 1 minute and then cooled. Then, 2 ml 1N sodium hydroxide solution (Appendix, p.iii) was added to maintain the pH at 12-13. After the addition of 1-2 drops of ammonium perpurate indicator it was titrated slowly with 0.01M EDTA and calculated as follows.

$$Ca\ (mg\ l^{-1}) = \frac{A \times B \times 400.8}{ml\ sample}$$

where A = ml titration for sample

B = mg $CaCO_3$ equivalent to 1.0 ml EDTA titrant at the calcium indicator end point

Total hardness

50ml of sample was taken in conical flask and 1ml ammonium chloride-ammonium hydroxide buffer solution was added. After the addition of 100mg Erichrome Black T indicator it was titrated against 0.01M, EDTA solution.

$$Hardness\ as\ mg\ l^{-1}\ CaCO_3 = \frac{ml\ EDTA\ used \times 1000}{ml\ sample}$$

Magnesium

It was estimated from EDTA and hardness titration (taken from hardness estimation).

Mg (mg l^{-1}) = Total hardness (as mg $CaCO_3$ l^{-1}) – Calcium hardness × 0.244 (as mg $CaCO_3$ l^{-1})

Chloride

50 ml water sample was taken in a flask and 2 ml of potassium chromate indicator was added. The contents of flask were titrated against 0.02 N silver nitrate solution chloride concentration in the sample was calculated as follows.

$$Chloride\ (mg\ l^{-1}) = \frac{(ml \times N)\ of\ AgNO_3 \times 1000 \times 35.5}{ml\ of\ sample}$$

Carbonates and bicarbonates

Estimation of carbonates and bicarbonates was done following the methods of Richards (1954). 50 ml water sample was taken in a clear flask. To this 5 drop phenolphthalein indicator was added. The appearance of pink colour indicated the presence of carbonates. Then it was titrated against 0.01 N sulphuric acid till the solution turned colourless To above solution 2 drops of methyl orange indicator were added. It was again titrated against 0.01 N sulphuric acid till the colour charged from yellow to rose red. This indicated bicarbonate presence.

$$\text{Carbonate (meq l}^{-1}) = 2y \times \text{normality of } H_2SO_4 \times \frac{1000}{\text{ml of aliquot}}$$

$$= 2y \times 2$$

$$\text{Bicarbonate (meq l}^{-1}) = (z - 2y) \times \text{normality of } H_2SO_4 \times \frac{1000}{\text{ml of aliquot}}$$

where y = reading of burette for titration of carbonates

z = reading of burette for titration of bicarbonates

Photosynthetic rate and stomatal conductance

The photosynthetic rate and stomatal conductance of fully expanded leaves of plants were was measured at 50, 70 and 90 DAS (days after sowing) i.e. at vegetative, flowering and fruiting stages respectively. It was measured using Li-COR 6200 portable photosynthesis system. The leaves of approximately same age and size were selected and measurement of photosynthesis was done at 1100 µmol $m^{-2}s^{-1}$ and photosynthetic active radiations at 1100-1200 hrs. The observations were replicated thrice and data were was recorded.

Photosynthetic water use efficiency

It was calculated by using data of photosynthetic rate and stomatal conductance (Das *et al.*, 1999) as follows.

$$\text{PWUE} = \frac{\text{Photosynthetic rate}}{\text{Stomatal conductance}}$$

EXPERIMENTAL RESULTS

Experiment I

Photosynthetic rate

CWW proved beneficial in enhancing the photosynthetic rate at all the growth stages studied. 100% CWW proved better than 50% CWW and GW by showing an increase of 8.79%, 8.63% and 6.68% over control at vegetative, flowering and fruiting stages respectively (Table 4). Crop also performed well under 50% CWW when compred with GW and recorded an increase of 4.09%, 3.82% and 3.28%. Among various doses of nitrogen, N_{120} proved effective as it recorded an increase of 45.63%, 37.61% and 32.40% over N_0 (Table 4). Rest of the doses of nitrogen i.e. N_{80} and N_{40} also enhanced the photosynthesis but proved deficient showing the importance of nitrogen in this most important metabolic activity of plant. Regarding interactions 100% CWW×N_{120} significantly increased the photosynthetic rate and recorded an increase of 57.25%, 48.34% and 40.04% over GW×N_0 at all the sampling stages. It may be pointed out that the higher dose of nitrogen significantly increased the photosynthetic rate when interacted with 100% CWW, 50% CWW and GW as compared to other doses of nitrogen. The increasing trend was observed with the increase in nitrogen. While observing the response of crop, increasing trend was observed in photosynthetic rate upto the flowering stage only thereafter it started decreasing.

Stomatal conductance

Stomatal conductance was significantly increased by CWW at vegetative and flowering stage, whereas at fruiting stage it proved non-significant. At vegetative stage 100% CWW showed an increase of 3.08% over control whereas 50% CWW was at par with GW (Table 5). At flowering stage 100% CWW showed an increase of 2.50% over control but it was at par with 50% CWW which in turn was at par with GW, thus showing suitable

Table 4. Effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) on photosynthetic rate (μ mol (CO_2) $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of nitrogen at three growth stages

	50 DAS				70 DAS			
Treatments	GW	50% CWW	100% CWW	Mean	GW	50% CWW	100% CWW	Mean
N_0	15.44	15.91	16.43	15.93	19.01	19.46	20.00	19.49
N_{40}	17.30	17.94	18.86	18.03	21.73	22.53	23.42	22.56
N_{80}	19.54	20.41	21.37	20.44	24.70	25.93	27.31	25.98
N_{120}	22.12	23.18	24.28	23.19	25.63	26.63	28.20	26.82
Mean	18.60	19.36	20.24		22.77	23.64	24.73	

	90 DAS				CD at 5%			
Treatments	GW	50% CWW	100% CWW	Mean		50 DAS	70 DAS	90 DAS
N_0	16.91	17.31	17.72	17.31	Water	0.024	0.024	0.025
N_{40}	19.11	19.72	20.40	19.74	Nitrogen	0.033	0.034	0.029
N_{80}	21.43	22.28	23.14	22.28	Interaction	0.047	0.048	0.050
N_{120}	22.17	22.92	23.68	22.92				
Mean	19.91	20.56	21.24					

N.B: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{30} kg ha^{-1} (0.082g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied basally.

Table 5. Effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) on stomatal conductance (m mol $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of nitrogen at three growth stages

Treatments	50 DAS				70 DAS			
	GW	50% CWW	100% CWW	Mean	GW	50% CWW	100% CWW	Mean
N_0	0.342	0.349	0.356	0.349	0.364	0.369	0.375	0.369
N_{40}	0.355	0.360	0.367	0.361	0.388	0.391	0.397	0.392
N_{80}	0.363	0.367	0.373	0.368	0.401	0.406	0.412	0.406
N_{120}	0.400	0.404	0.409	0.404	0.406	0.409	0.414	0.410
Mean	0.365	0.370	0.376		0.390	0.394	0.400	

Treatments	90 DAS				CD at 5%			
	GW	50% CWW	100% CWW	Mean		50 DAS	70 DAS	90 DAS
N_0	0.358	0.363	0.367	0.363	Water	0.0048	0.0052	NS
N_{40}	0.375	0.378	0.383	0.379	Nitrogen	0.0055	0.0060	0.0074
N_{80}	0.389	0.392	0.396	0.392	Interaction	NS	NS	NS
N_{120}	0.390	0.394	0.398	0.394				
Mean	0.378	0.382	0.386					

N.B: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{30} kg ha^{-1} (0.082g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied basally.

consumption of WW for this parameter. Among various doses of nitrogen, N_{120} recorded an increase of 15.85%, 10.92% and 8.61% over N_0 at vegetative, flowering and fruiting stages respectively. However at flowering and fruiting stages, N_{80} was at par with N_{120}. It may be pointed out that N_{80} proved optimum dose, as it significantly increased the stomatal conductance at flowering and fruiting stages and N_{120} showed luxury consumption. N_{40} also enhanced above mentioned parameter in comparison to N_0 but proved deficient. Interaction proved non-significant at all sampling stages. Like photosynthetic rate, stomatal conductance upto flowering stage also increased and then it started decreasing.

Photosynthetic water use efficiency

This parameter was also improved by the application of 100% CWW at all the three growth stages and it showed an increase of 5.49%, 5.90% and 4.56% over GW (Table 6). PWUE also increased under 50% CWW recording an increase of 2.65%, 2.73% and 2.31% over control. Regarding various doses of nitrogen, N_{120} effectively increased the PWUE by registering an increase of 25.67%, 24.07% and 21.92% over N_0 (Table 6). Rest of the doses of nitrogen i.e. N_{80} and N_{40} were regarded as deficient although these doses also enhanced this parameter when compared with N_0. Interaction effect proved non-significant. This parameter also showed increasing trend with increasing levels of nitrogen. PWUE increased from vegetative to flowering stage and decreased from flowering to fruiting stage.

Experiment II

Photosynthetic rate

CWW treatment significantly enhanced photosynthetic rate at all the growth stages. 100% CWW showed an increase of 7.84%, 7.25% and 5.64% over GW at vegetative, flowering and fruiting stages respectively (Table 7). Photosynthetic rate also showed an increase of 3.68%, 3.31% and 2.68% over control with 50% CWW. Among various doses of phosphorus, P_{60} increased photosynthetic rate and recorded an increase of 39.31%, 36.84% and 35.20%

Table 6. Effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) on photosynthetic water use efficiency (μ mol mol^{-1}) of *Brassica juncea* grown with four different doses of nitrogen at three growth stages

Treatments	50 DAS				70 DAS			
	GW	50% CWW	100% CWW	Mean	GW	50% CWW	100% CWW	Mean
N_0	45.14	45.58	46.15	45.63	52.22	52.73	53.33	52.76
N_{40}	48.73	49.83	51.38	49.98	56.00	57.62	58.99	57.54
N_{80}	53.82	55.61	57.29	55.58	61.59	63.86	66.28	63.91
N_{120}	55.30	57.37	59.36	57.34	63.12	65.11	68.11	65.46
Mean	50.76	52.10	53.54		58.24	59.83	61.68	

Treatments	90 DAS				CD at 5%			
	GW	50% CWW	100% CWW	Mean		50 DAS	70 DAS	90 DAS
N_0	47.23	47.68	48.28	47.71	Water	0.723	0.877	0.985
N_{40}	50.96	52.16	53.26	52.13	Nitrogen	0.835	1.013	1.138
N_{80}	55.08	56.83	58.43	56.79	Interaction	NS	NS	NS
N_{120}	56.84	58.17	59.49	58.17				
Mean	52.50	53.71	54.89					

N.B: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of P_{30} kg ha^{-1} (0.082g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied basally.

Table 7. Effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) on photosynthetic rate (μ mol (CO_2) $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of phosphorus at three growth stages

Treatments	50 DAS				70 DAS			
	GW	50% CWW	100% CWW	Mean	GW	50% CWW	100% CWW	Mean
P_0	14.73	15.14	15.61	15.16	17.89	18.33	18.85	18.36
P_{20}	16.98	17.59	18.26	17.61	19.84	20.51	21.26	20.54
P_{40}	19.13	20.06	20.91	20.03	21.78	22.65	23.56	22.66
P_{60}	20.35	21.02	21.99	21.12	24.22	25.01	26.13	25.12
Mean	17.80	18.45	19.19		20.93	21.63	22.45	

Treatments	90 DAS				CD at 5%			
	GW	50% CWW	100% CWW	Mean		50 DAS	70 DAS	90 DAS
P_0	15.90	16.18	16.55	16.21	Water	0.029	0.027	0.029
P_{20}	17.62	18.18	18.77	18.19	Phosphorus	0.034	0.031	0.033
P_{40}	20.30	21.01	21.72	21.01	Interaction	0.059	0.053	0.057
P_{60}	21.42	21.89	22.44	21.92				
Mean	18.81	19.32	19.87					

N.B: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of N_{80} kg ha^{-1} (0.076g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied basally.

over control (Table 7). Under rest of the doses i.e. P_{40} and P_{20} plants also showed better response than P_0 but were deficient. Regarding interaction effect 100% CWW x P_{60} significantly enhanced photosynthetic rate and gave an increase of 49.29%, 46.06% and 41.13% over GW x P_0 at all the three sampling stages. 60 kg P ha^{-1} gave better results when interacted with 100% CWW and 50% CWW, even with GW it performed better. It was noted that photosynthetic rate decreased with decreasing doses of phosphorus. Photosynthetic rate increased upto flowering stage and then decreased.

Stomatal conductance

100% CWW significantly increased stomatal conductance at vegetative and flowering stages showing an increase of 3.42% and 3.20% where at fruiting stage it proved non significant. Comparatively lesser increase was recorded by 50% CWW. It recorded an increase of 1.61% and 1.39% only at vegetative and flowering stages, showing importance of wastewater, but at vegetative and flowering stages. At vegetative stage 50% CWW was at par with GW, whereas at flowering stage, on the one hand 50% CWW was at par with 100% CWW and it was at par with GW on the other (Table 8). P_{60} proved efficacious and recorded an increase of 13.01%, 11.87 and 10.95% at vegetative, flowering and fruiting stages. P_{40} and P_{20} also performed better than control i.e. P_0. It was noted that stomatal conductance also started decreasing with decreasing levels of phosphorus. It showed an increasing trend from vegetative to flowering stage only.

Photosynthetic water use efficiency

CWW enhanced PWUE at vegetative and fruiting stages, whereas at flowering stage it proved non-significant. 100% CWW showed an increase of 3.97% and 3.21% over GW respectively at vegetative and fruiting stages. 50% CWW also marginally increased PWUE showing 1.70% and 1.51% increase at vegetative and fruiting. At vegetative stage 50% was at par with GW (Table 9). At fruiting stage 50% CWW was at par with GW on one hand whereas on the other hand it was at par with 100% CWW.

Table 8. Effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) on stomatal conductance (m mol $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of phosphorus at three growth stages

Treatments	50 DAS				70 DAS			
	GW	50% CWW	100% CWW	Mean	GW	50% CWW	100% CWW	Mean
P_0	0.336	0.343	0.351	0.343	0.354	0.360	0.367	0.348
P_{20}	0.347	0.353	0.361	0.354	0.369	0.374	0.381	0.375
P_{40}	0.366	0.372	0.378	0.372	0.386	0.392	0.397	0.392
P_{60}	0.384	0.388	0.392	0.388	0.400	0.404	0.409	0.404
Mean	0.358	0.364	0.371		0.377	0.383	0.389	

Treatments	90 DAS				CD at 5%			
	GW	50% CWW	100% CWW	Mean		50 DAS	70 DAS	90 DAS
P_0	0.348	0.352	0.359	0.353	Water	0.0064	0.0072	NS
P_{20}	0.360	0.365	0.370	0.365	Phosphorus	0.0074	0.0083	0.0086
P_{40}	0.373	0.378	0.382	0.377	Interaction	NS	NS	NS
P_{60}	0.389	0.392	0.394	0.392				
Mean	0.368	0.371	0.376					

N.B: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of N_{80} kg ha^{-1} (0.076g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied basally.

Table 9. Effect of ground water (GW), 50% city wastewater (CWW) and 100% city wastewater (CWW) on photosynthetic water use efficiency (μ mol mol^{-1}) of *Brassica juncea* grown with four different doses of phosphorus at three growth stages

Treatments	50 DAS				70 DAS			
	GW	50% CWW	100% CWW	Mean	GW	50% CWW	100% CWW	Mean
P_0	43.83	44.13	44.47	44.14	50.53	50.91	51.36	50.93
P_{20}	48.93	49.83	50.58	50.00	53.76	54.83	55.80	54.86
P_{40}	52.26	53.92	55.31	53.87	56.42	57.78	59.34	57.85
P_{60}	52.99	54.17	56.09	54.42	60.55	61.90	63.88	61.00
Mean	49.67	50.51	51.64		55.32	56.36	56.81	

Treatments	90 DAS				CD at 5%			
	GW	50% CWW	100% CWW	Mean		50 DAS	70 DAS	90 DAS
P_0	45.68	45.96	46.10	45.92	Water	0.967	NS	1.089
P_{20}	48.94	49.80	50.72	49.82	Phosphorus	1.116	2.262	1.258
P_{40}	54.42	55.58	56.85	55.62	Interaction	NS	NS	NS
P_{60}	55.06	55.84	56.95	55.96				
Mean	51.03	51.80	52.67					

N.B: Subscript values denote the amount of nitrogen (N) in kg ha^{-1}. A uniform dose of N_{80} kg ha^{-1} (0.076g kg^{-1} soil) and K_{30} kg ha^{-1} (0.027g kg^{-1} soil) was applied basally.

Regarding various doses of phosphorus P_{40} proved optimum, as it was at par with P_{60} at vegetative and fruiting stage and recorded an increase of 22.03% and 21.12% over GW. Whereas at flowering stage P_{60} differed significantly from other doses and showed an increase of 19.76% over GW. Water use efficiency also showed an increasing trend from vegetative to flowering stage.

DISCUSSION

Crop yields are generally limited by three major macro-nutrients NPK, since these are removed by crops comparatively in larger quantity. Soil fertility can therefore, be maintained by supplying these nutrients as fertilizers timely and most importantly in adequate quantities. In the present study, in addition to inorganic fertilizers where nitrogen and phosphorus were given in different doses alongwith K as fixed dose. Crop was irrigated with city wastewater which also contained NPK in addition to Ca, Mg and Cl^- in various amounts. NPK ~~has~~ a well established role in cell division, elongation, expansion and differentiation as also in biochemical reactions (Gardner *et al.*, 1985). In both the experiments one of the most important physiological parameter, photosynthetic rate was observed alongwith stomatal conductance and PWUE and these parameters almost responded linearly to the increasing doses of nitrogen and phosphorus. In Experiment I, N_{120} with 100% C WW proved optimum for photosynthetic rate and PWUE, while maximum stomatal conductance was observed at N_{80} showing the luxury consumption of nitrogen (Figs. 1-3). It may be mentioned that chlorophyll molecule and other enzymes and co-enzymes involved in photosynthesis being themselves nitrogenous in nature depend upon this element for their production (Devlin and Witham, 2001) and in green leaves 75% nitrogen is located in the chloroplasts. Similarly Rubisco an enzyme which helps RUBP accept CO_2 leading to the formation of 3 phosphoglycerate, the first stable compound of dark reaction of photosynthesis in C_3 plants contains 20-30% nitrogen whereas in C_4 plants it may be about 10%. Therefore this element is essential for photosynthesis as observed in the present study where photosynthetic rate was increased with increasing doses of nitrogen. It is also involved in increased growth and leaf area (Gardner *et al.*, 1985) thus allowing the plants to trap maximum solar radiations.

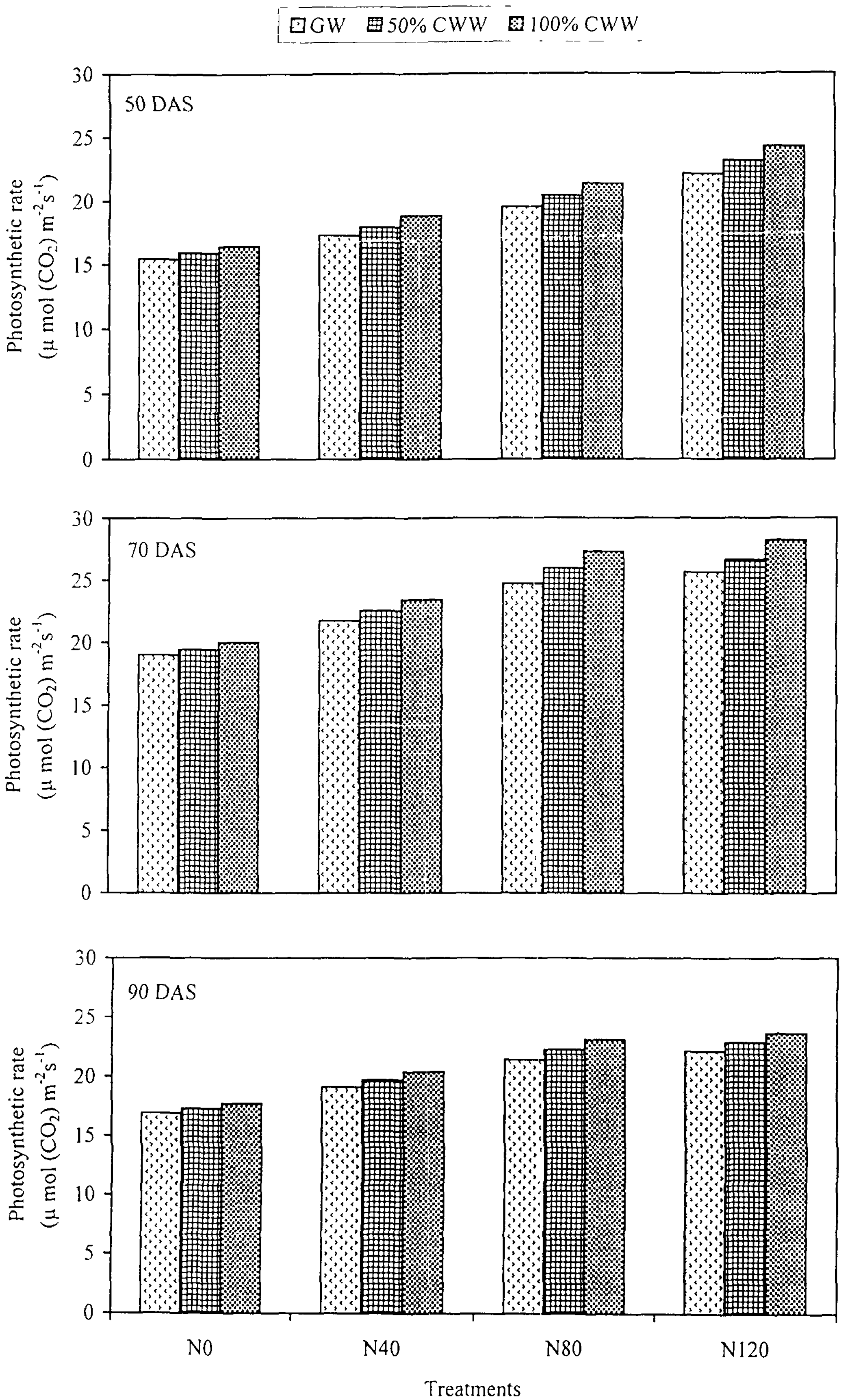

Fig. 1. Effect of ground water (GW), 50% CWW and 100% CWW on photosynthetic rate (μ mol (CO_2) $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of nitrogen at three growth stages.

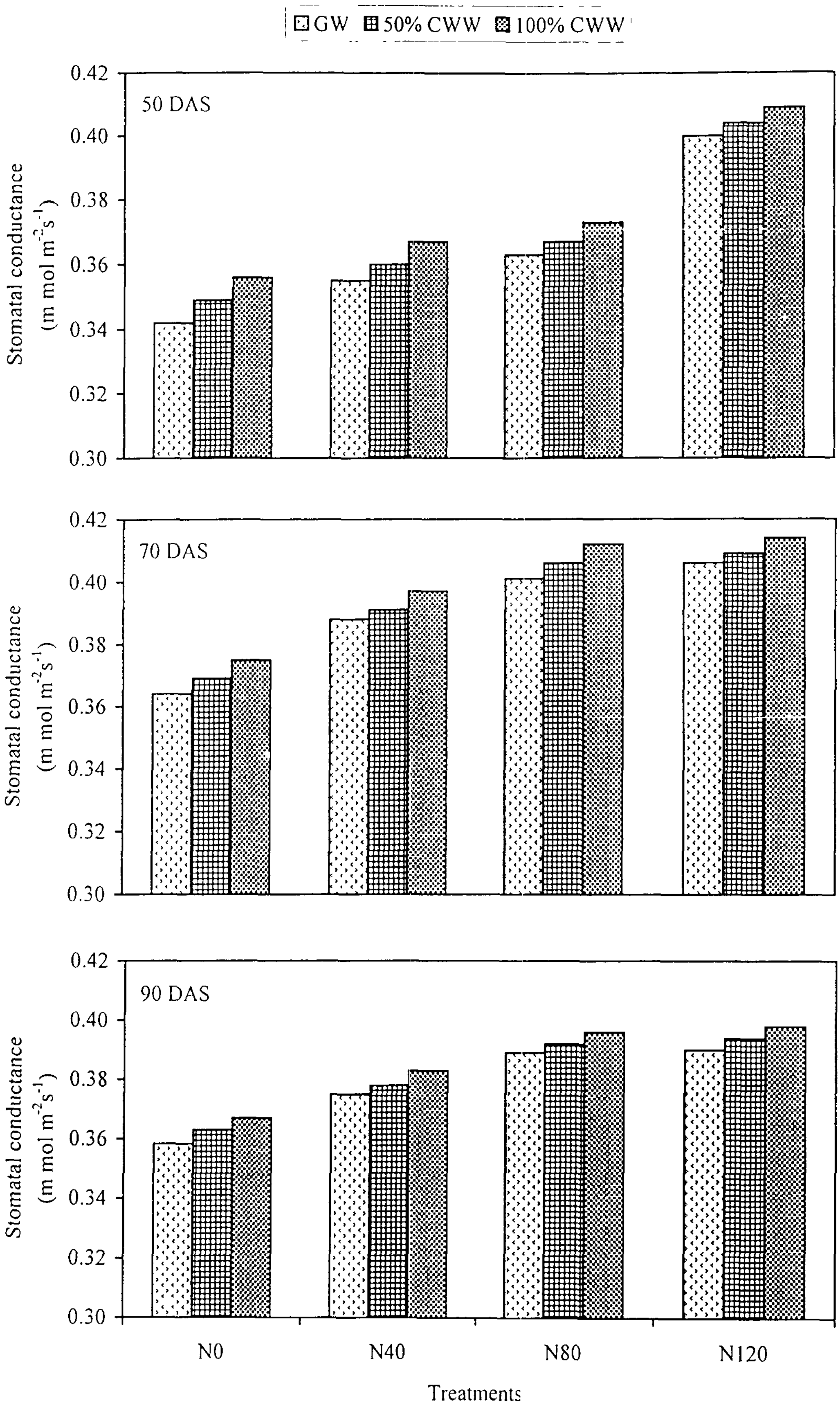

Fig. 2. Effect of ground water (GW), 50% CWW and 100% CWW on stomatal conductance (m mol $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of nitrogen at three growth stages.

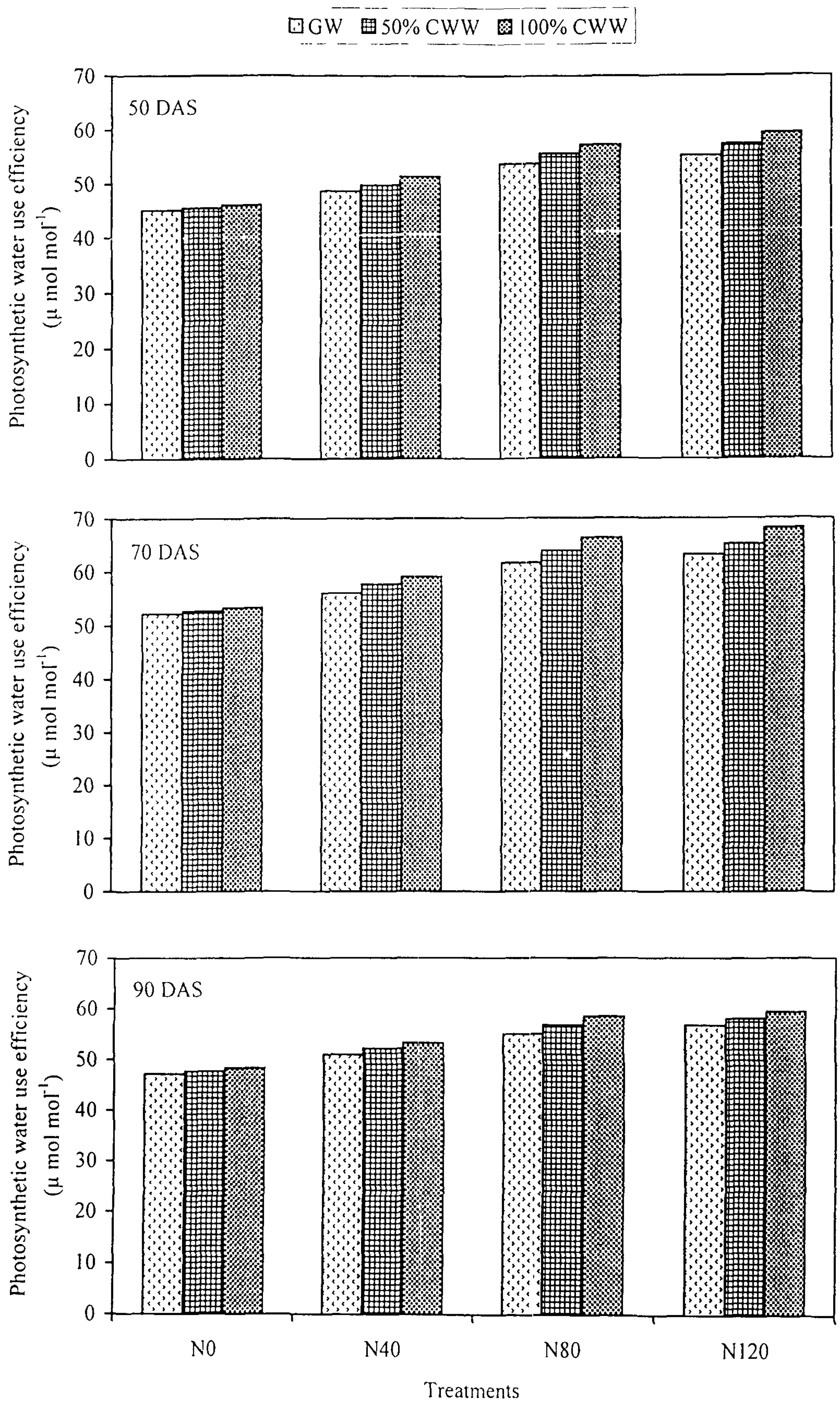

Fig. 3. Effect of ground water (GW), 50% CWW and 100% CWW on photosynthetic water use efficiency (μ mol mol^{-1}) of *Brassica juncea* grown with four different doses of nitrogen at three growth stages.

While considering the phosphorus (Experiment II), P_{60} with 100% CWW enhanced the photosynthetic rate and stomatal conductance, however for PWUE, P_{40} along with 100% CWW proved optimum (Figs. 4-6). Since phosphorus being an essential component of ATP and NADPH which are the products of light reaction of photosynthesis, strongly affects the photosynthesis and carbon partitioning in light-dark reactions. In the light, for optimal photosynthesis in chloroplast Pi concentrations in the range of 2.0-2.5 mM. seems to be required and photosynthesis is totally inhibited when Pi concentration falls below 1.4-1.0mM (Robinson and Giersch, 1987). Likewise Lauer *et al.* (1989a) have also observed that some reactions of photosynthesis like carboxylation were impaired due to phosphorus deficiency. Its deficiency also reduces the leaf expansion, leaf surface area and number of leaves in soybean as observed by Freeden *et al.* (1989), Lynch *et al.* (1991) due to which photosynthetic rate decreased considerably.

Since potassium was also given as a basal dose uniformly with all treatments and it was also present in wastewater therefore this nutrient could have enhanced the photosynthesis. It may be pointed out that potassium being the dominant counterion to the light induced H^+ flux across thyllakoid membranes (Tester and Blatt, 1986) maintains transmembrane pH gradient necessary for ATP synthesis (photophosphorylation) in analogy to ATP synthesis in mitochondria. Therefore, K supply can increase the leaf area and chlorophyll leaf area^{-1}, RUBP carboxylase activity as well as photorespiration due to stronger depletion of CO_2 at catalytic sites of enzyme, thus leading to increase in photosynthetic rate. Potassium nutritional status may also affect photosynthesis via its regulation in stomatal conductance.

In addition to these macro-nutrients wastewater also contained some other nutrients like Mg and Cl^- which have various direct or indirect roles in photosynthesis. Mention may be made of Mg^{2+} which is the central atom of chlorophyll molecule and is also required by PEP carboxylase, an enzyme

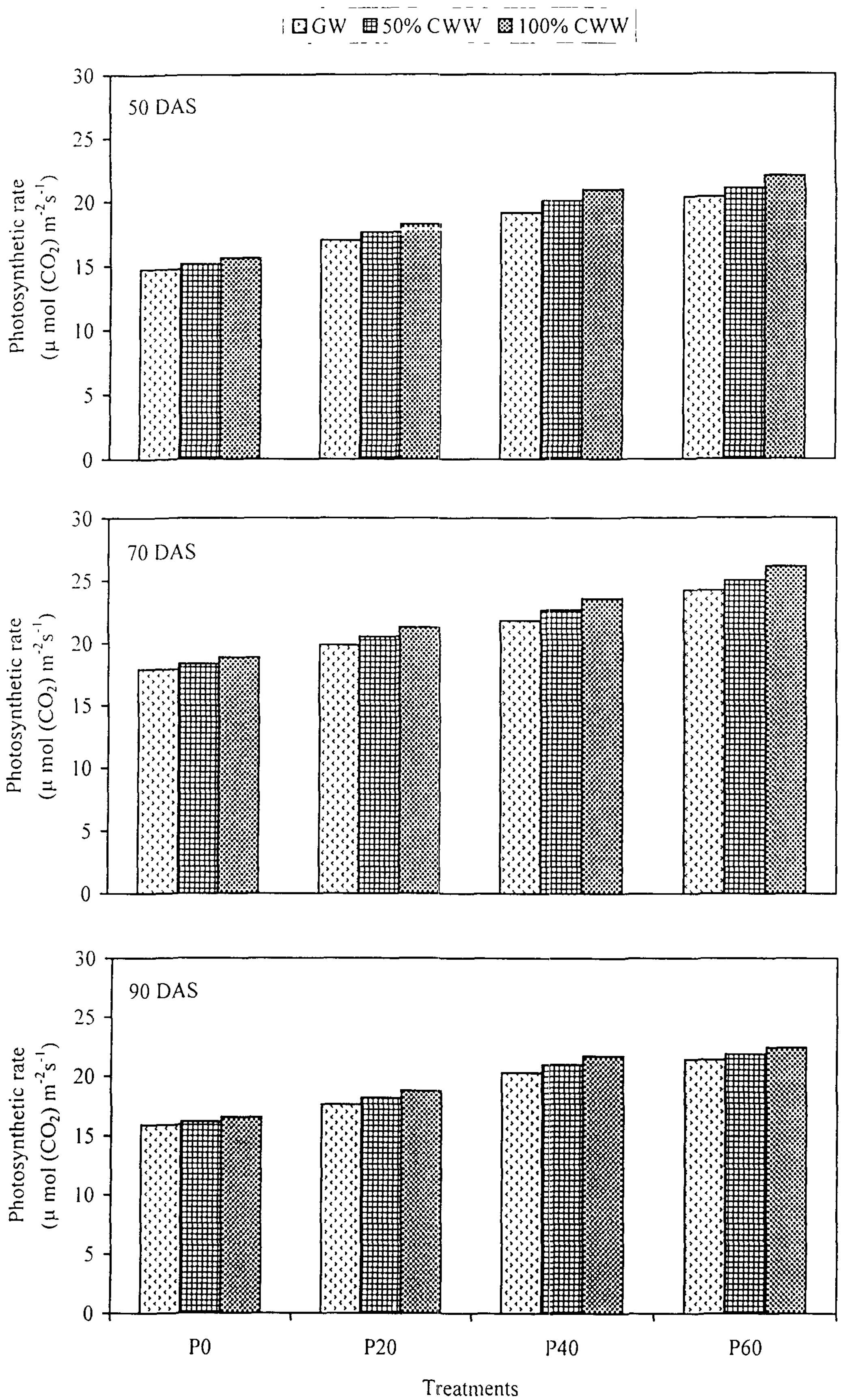

Fig. 4. Effect of ground water (GW), 50% CWW and 100% CWW on photosynthetic rate (µ mol (CO_2) $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of phosphorus at three growth stages.

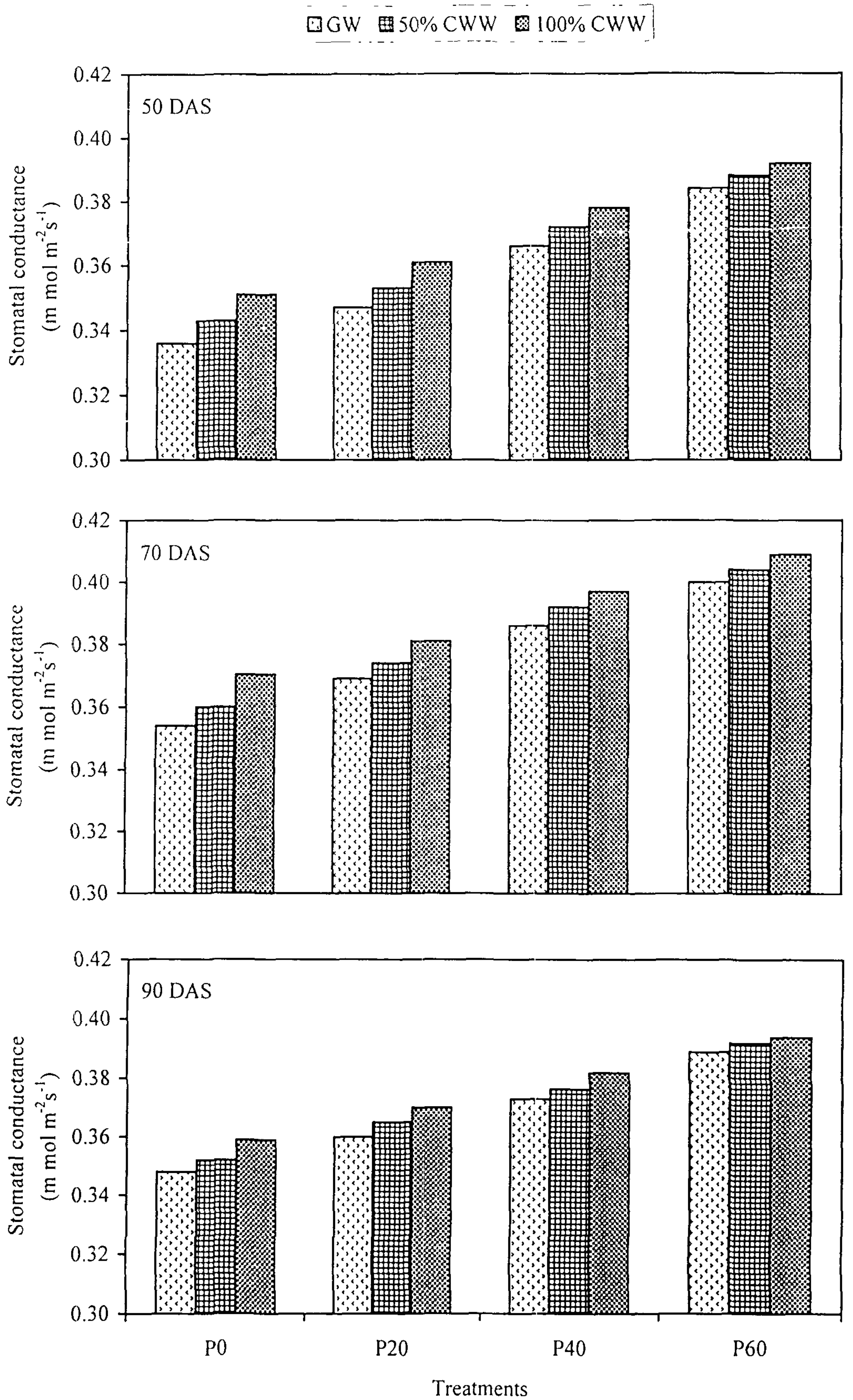

Fig. 5. Effect of ground water (GW), 50% CWW and 100% CWW on stomatal conductance (m mol $m^{-2}s^{-1}$) of *Brassica juncea* grown with four different doses of phosphorus at three growth stages.

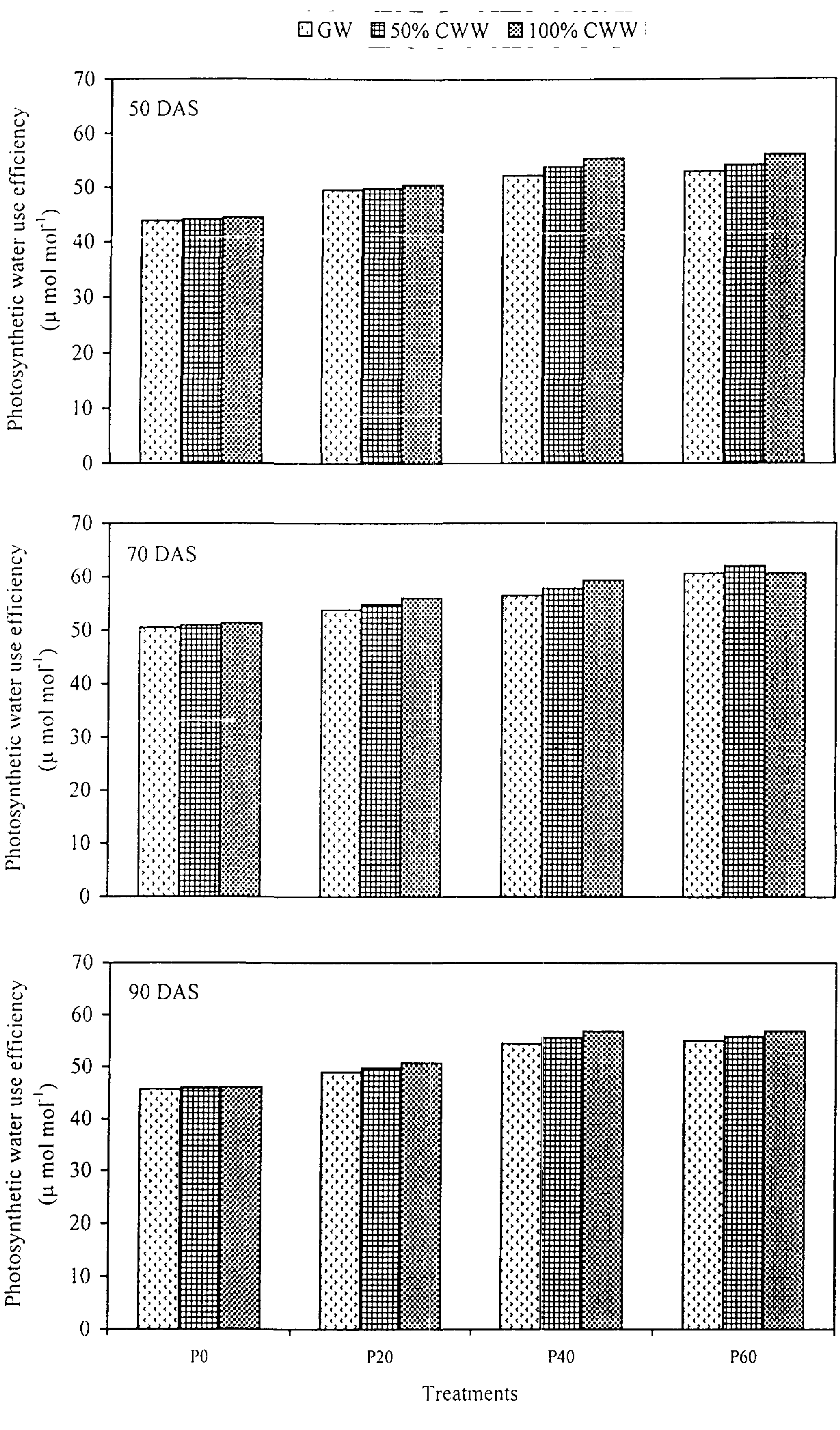

Fig. 6. Effect of ground water (GW), 50% CWW and 100% CWW on photosynthetic water use efficiency (μ mol mol^{-1}) of *Brassica juncea* grown with four different doses of phosphorus at three growth stages.

essential in CO_2 fixation of C_4 plants. Furthermore, Rubico is also strongly activated by Mg^{2+}. As calculated on both leaf area and unit chlorophyll, photosynthetic rate is lower in the leaves of Mg^+ deficient plants (Marschner, 2004). Chlorine being an essential component of stomatal regulation because opening and closure of stomata is mediated by fluxes of potassium and accompanying anions such as malate and chloride. Chlorine plays a fundamental role in water splitting system of PSII of photosynthesis. In chlorine deficient plants reduction was observed in dry weight and leaf size and interveinal chlorosis thereby affecting the photosynthetic rate in mature leaf blades (Marschner, 1995).

In both the experiments, photosynthetic rate increased upto flowering stage, after which it started decreasing, as leaves grow, their ability to photosynthesize increases for a time and often even before maturity, begins slowly to decrease, because old senescent leaves eventually become yellow and are unable to photosynthesize due to chlorophyll breakdown and loss of functional chloroplasts (Salisbury and Ross, 1995)

Conclusions

1. The present study clearly indicates that 100% CWW is best suited for higher photosynthetic rate of *Brassica juncea* var. *Varuna* when compared with 50% WW and GW.
2. With the help of these parameters we can judge the performance of mustard for other parameters also and a good yield can be expected because yield is a manifestation of several morphological and physiological characteristics of a crop.
3. Nitrogen was comparatively more effective in comparison with phosphorus.
4. Photosynthesis enhanced upto 70d stage while decreased at 90d stage in both experiments.

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APPENDIX

APPENDIX

Alkali iodide azide reagent :- 50g sodium hydroxide and 15g potassium iodide diluted to 100ml with DDW. 1g sodium azide dissolved in 4ml of DDW and added to the above solution.

Ammonium acetate solution :- Dilute 57ml glacial acetic acid to 800ml with DDW and neutralize to pH 7.0 with concentrated ammonium hydroxide and final volume was made upto 1000ml.

Ammonium chloride ammonium hydroxide buffer:- (a) 16.9 g ammonium chloride dissolved in 143 ml concentrated ammonium hydroxide (b) 1.179 g of dissolved EDTA and 0.780 g magnesium sulphate dissolved in 50 ml DDW. Both (a) and (b) were mixed and diluted to 250 ml with DDW.

Ammonium molybdate solution (2.5%):- (a) 25.0g ammonium molybdate dissolved in 175ml DDW (b) Add 280ml concentrated H_2SO_4 to 400ml DDW and cool. Mix the two solutions (a) and (b) and final volume made upto 1 litre with DDW.

Ammonium purpurate :- 150mg ammonium purpurate dissolved in 100g ethylene glycol.

Brucine sulfanilic acid solution :- 60mg sulfanilic acid dissolved in 70ml warm DDW. After addition of 20ml concentrated HCl, the volume was made upto 100ml.

Conditioning reagent :- 50ml of glycerol mixed in a solution containing 30ml concentrated HCl + 300ml DDW + 100ml 95% ethyl alcohol and 75g sodium chloride.

Dickman and Bray's reagent :- 15g ammonium molybdate dissolved in 300ml DDW (about 60°C) cooled and filtered, if necessary. To this, 400ml 10N HCl was added and final volume was maintained at 1000ml with DDW.

Diphenyl amine indicator :- 0.5g diphenyl amine dissolved in a mixture of 20ml DDW and 100ml concentrated H_2SO_4.

EDTA (0.01M) :- 3.723g of sodium salt EDTA dissolved in DDW and diluted to 1000ml.

Erichrome black T indicator :- 0.4g erichrome black T grind with 100g powdered sodium chloride.

Ferrous ammonium sulphate (0.5N) :- 196g hydrated ferrous ammonium sulphate dissolved in DDW, to this 20ml concentrated H_2SO_4 was added and the final volume made upto 1000ml.

Hydrochloric acid (0.2N) :- 17.24ml HCl mixed with DDW and diluted upto 1000ml.

Liquid ammonia (1:1) :- Ammonia having 0.88 specific gravity diluted with equal volume of DDW.

Manganous sulphate solution :- 100g manganous sulphate dissolved in warm DDW and volume made upto 200ml.

Methyl orange indicator (0.05%):- 0.5g methyl orange dissolved in 100ml DDW.

Murexide indicator :- 0.2g ammonium purpurate mixed with 100g powdered sodium chloride.

Olsen's reagent :- 42.0g sodium bicarbonate dissolved in DDW to give 1 liter solution. The pH was adjusted to 8.5 with the addition of small amount of sodium hydroxide.

Phenol disulphonic acid :- This was prepared by taking 25g pure phenol (C_6H_5OH, crystal white) in a conical flask (500ml) to which 150ml concentrated H_2SO_4 and 75ml fuming sulphuric acid were added and kept on boiling water bath for 2 hours covered with glass. After cooling, it was stored in an amber coloured bottle.

Phenolphthalein indicator :- 0.5 gms phenolphthalein dissolved in 50 ml DDW, add 0.05N CO_2 free NaOH solution dropwise till the solution turns faintly pink.

Potassium chromate indicator :- 5g potassium chromate (K_2CrO_4) dissolved in DDW and final volume was made to 100 ml.

Potassium dichromate (1N) :- 49.04g potassium dichromate dissolved in 1000 ml of DDW.

Sodium hydroxide solution (0.1N) :- 4g sodium hydroxide dissolved in

1000ml DDW.

Sodium hydroxide solution (1N) :- 4g NaOH dissolved in DDW final volume made upto 100ml.

Sodium thiosulphate (0.25N) :- 6.2g sodium thiosulphate dissolved in 1000ml DDW.

Standard ferrous ammonium sulphate solution (0.1N) :- 39.2g ferrous ammonium sulphate dissolved in DDW. 20ml of concentrated sulphuric acid was added and volume made upto 1000ml.

Standard silver nitrate titrant (0.02N) :- 3.400g silver nitrate dissolved in DDW and final volume made 1000ml.

Stannous chloride solution :- 10g crystalline stannous chloride dissolved in 25ml concentrated HCl by warming then stored in an amber coloured bottle, giving 40% stannous chloride stock solution. Just before use. 0.5ml was diluted to 66ml with DDW.

Starch indicator :- 1g starch dissolved in 100ml warm (80-90°C) DDW and few drops of formaldehyde solution was added.

Sulphuric acid (0.01N) :- 0.272ml sulphuric acid diluted in DDW and final volume made 1 litre.

Sulphuric acid (7N) :- 190.4 ml concentrated sulphuric acid added to DDW and the final volume made upto 1000ml.